A Study of the Reproductive Cycle in the California Acmaeidae (Gastropoda)

Part I

by

HARRY K. FRITCHMAN II

(From the Department of Zoology, University of California, Berkeley, California, and the Department of Biology, Boise Junior College, Boise, Idaho)

(Plate 10)

INTRODUCTION

The limpets of the genus Acmaea Eschscholtz, 1833, are one of the most common gastropod types to be found in the rocky intertidal zone of the Pacific Coast of the United States. They are conspicuous not only because of the large number of individuals which are present but also because of the numerous species encountered. These various species and subspecies, of which 21 are known within the boundaries of California, have occupied practically every ecologic niche available to them in the rocky intertidal zone. The requirements of such a niche seem to be essentially three in number: a fairly rigid substrate for attachment of the broad foot, a source of vegetable food and at least some contact periodically with sea water. Wherever these conditions are met, there will usually be found a species of Acmaea which has become adapted to the niche. These niches may be quite unspecific and several eurytopic species may inhabit what seems to be the same type of habitat. By contrast, there are several very stenotopic species which are restricted to specific algae or other very special locations. It is evident that an extensive radiation has taken place in this genus, allowing its species to colonize to a remarkable degree this rocky intertidal zone. It is the intent of this work to elucidate the reproductive mechanisms and cycles of the several species of this genus and, in so doing, to suggest what the nature of these speciation processes might have been.

The Acmaeidae are a relatively primitive family of aspidobranch gastropods, containing only three genera in addition to Acmaea. All of the members of the genus Acmaea are superficially bilaterally symmetrical animals bearing a cap-like shell. Internally however, they exhibit to a high degree the effects of torsion. The anatomy of A. fragilis has been described by Willcox (1898), and some of the salient features of the genus are noted in the following. A single bipectinate ctenidium is present, the post-torsional left, which projects diagonally forward and across the mantle cavity. The anus opens into the right side of the mantle cavity and is flanked on each side by the apertures of the renal organs. As in all aspidobranchs, the post-torsional right kidney has been greatly developed while the left has become much reduced. The right post-torsional gonad is the functional gonad, a feature common to all gastropods. The latter organ opens into the distal end of the right kidney and the genital products are thus discharged via the right renal aperture. There has been some disagreement in the literature concerning the actual method of shedding the gametes. Willcox (op. cit.) postulates that the right renal papilla containing the renal aperture may serve as a copulatory organ in males, thus permitting internal fertilization. Indeed, she describes (Willcox, 1905) what might be interpreted as a mating procedure between a pair of A. testudinalis. Thorson (1935) found that A. rubella contained larvae in the mantle cavity. These are the only descriptions in the literature which support the idea of internal fertilization. All other evidence is to the contrary and indicates that external fertilization is the rule, if not the only method employed by the Acmaeidae (Boutan 1898, Hewatt 1938, Thiele 1931). My observations certainly support this conclusion since, of the 11 species which have been investigated in this study, 10 have been known to spawn in the laboratory, the eggs and sperm being released freely into the water. Thus, barring the effects of proximity due either to active aggregation or simply to dense populations, fertilization of the eggs takes place at random in the sea water.

The only recent definitive work on Acmaea is a revision of the genus written in 1937 by Avery Ransom Grant as a doctoral thesis at the University of California. In addition to the taxonomy of the group, this extensive and unpublished paper includes much information on the distribution and ecology of the numerous species of this genus.

During her earlier studies of the ecology of the California forms, Test (1945) became much interested in the question of speciation in the genus and, while not entirely rejecting the isolating effects of geographical barriers, she subscribed very strongly to the concept of ecologic or sympatric speciation (Test, 1946). Briefly, it was her belief that a single eurytopic species, through the process of mutation, gave rise to extreme variants which were able to colonize habitats within the rocky intertidal zone which were not available to the parental species. These changes could be either morphological, being reflected in modifications of the shell for fitting specific substrates (e.g., algal stipes), or physiological, which would allow the animal to withstand greater extremes of temperature and desiccation encountered in higher intertidal zones. To these could be added also the behavioristic changes such as preferences for smooth versus rough rock substrates and nocturnal instead of diurnal activity patterns. Test then postulated that the genetic integrity of these mutant populations would be maintained by either close or internal fertilization and thus a reproductive barrier would be created between the new and the parental populations. Because of anatomical restrictions, internal fertilization is known to be impossible and because close fertilization is possible only in dense populations (and improbable in the cases of some very stenotopic species) some other mechanism must have been operating to establish the reproductive barrier

have been operating to establish the reproductive barrier required by the concept of sympatric speciation. It was felt that if, under the impress of new environmental factors, the reproductive cycle of the variant population could be so altered that it no longer coincided with that of the parental group, the necessary genetic isolation could be achieved. A cyclic isolation was then to be sought for between presumed parental and offshoot species, a cyclic difference which could be correlated with the differences in the ecology of the species concerned.

Species List

The genus Acmaea, as revised by Grant (1937), includes five subgenera based on the characteristics of the radular ribbon from which the teeth have been removed. For details on this procedure see Fritchman (1960). Of these five subgenera, three are known to occur on the coast of California.

The subgenus which she considers to be the most primitive is the subgenus Acmaea Dall, 1871, whose type species is A. mitra Eschscholtz, 1833. This group has a radula which lacks the lateral hooks or uncini and which possesses basal bodies of simple pattern which fit closely together. Only three of the California species are referable to this subgenus: A. mitra, the type species, A. funiculata (Carpenter, 1864), and A. depicta (Hinds, 1842), a stenotopic form restricted to the eelgrasses Zostera and Phyllospadix.

The type species of the second subgenus, Notoacmaea (Iredale, 1915), is Acmaea pileopsis, a species not occurring on the West Coast of North America. The radula of the members of this subgenus has a more complicated basal body structure but lacks uncini. The plates are characterized by several protruding processes which prevent the basal bodies from fitting well together. California species referable to this group include the following: A. scutum Eschscholtz, 1833, A. fenestrata fenestrata (Reeve, 1855), A. fenestrata cribraria Carpenter, 1857, A. persona Eschscholtz, 1833, A. insessa (Hinds, 1842), and A. paleacea Gould, 1851.

The third subgenus found in California is Collisella Dall, 1871. All members of this group possess a radula each of whose plates bears a pair of uncini on the lateral margins. The following species are placed in this group: Acmaea pelta Eschscholtz, 1833, the type species of the subgenus, A. asmi (Middendorf, 1847), A. scabra (Gould, 1846), A. ochracea Dall, 1871, A. instabilis (Gould, 1846), A. digitalis Eschscholtz, 1833, A. limatula Carpenter, 1864, A. limatula morchii Dall, 1878, A. triangularis (Carpenter, 1864), A. conus Grant, 1945, and A. paradigitalis Fritchman, 1960.

One other species which occurs in California, Acmaea rosacea Carpenter, 1864, is included by Test as a species inquirendae. On the basis of the radular characters it should probably be assigned to the subgenus Notoacmaea.

Because of their geographical distribution, some of these species and races could not be studied since the collecting areas were restricted to the region of San Francisco. For this reason, the following were not investigated: Acmaea conus, A. f. fenestrata, A. limatula, and A. depicta, all of which are found only south of the Monterey Peninsula. Another group of species was not investigated, either because of their relative scarcity in the local intertidal zone or their occurrence subtidally. This group included A. rosacea, A. triangularis, A. funiculata, and except for a few short studies, A. instabilis and A. ochracea. A. paradigitalis was undescribed at the time of the study. The remaining eleven species were studied for periods ranging from seven months to somewhat more than three years.

Collecting Areas

Four areas in the vicinity of San Francisco were regularly visited to procure the samples of limpets used in the investigation. Three of these lie to the south of San Francisco in San Mateo County and are located on the open coast.

The first of these is the locality known as Rockaway Beach. This beach is terminated at its southern end by a rocky point (37°30'25" N; 122°30' W) at the tip of which is a partially destroyed concrete breakwater. This structure and the south-facing portion of the point were the sites of extensive collections.

The second area is Moss Beach (37°31'30" N; 122°31' W), a locale composed primarily of hard sandstone reefs of rather uniform height which extend for about 100 yards seaward from the sand beach.

Pigeon Point (37°12'20" N; 122°24' W), the southernmost of the three areas, is a rocky point upon which a lighthouse has been constructed. The collecting area to the north of the building consists of massive sandstone with imbedded boulders arising from a bottom of gravel and sand. Coralline algae are plentiful and Acmaea mitra, which uses this for food, is thus relatively abundant here.

The single northern area utilized as a collecting site was the village of Marshalls, Marin County (38°09'45" N; 122°53'30" W) on the east shore of Tomales Bay. This is a shallow inlet, very long and narrow, the long axis running northwest to southeast. Since its mouth is constricted and the bay is protected by the peninsula which forms its western shore, heavy surf is lacking inside the bay. The water temperature is somewhat higher than that of the open ocean and the salinity may also be altered. Its beaches are mostly of gravel and mud and heavy growths of the familiar open coast algae are absent. It is here that Acmaea limatula morchii was obtained.

The method here adopted for designating intertidal heights is that set forth by Ricketts and Calvin (1952). This system divides the intertidal zone into four subzones, the highest being zone one, the lowest, zone four. Zone one extends from the level of standard mean high water, +5.0 feet, upwards to the limits of the beach. It is an area of dry rocks, little algae, and inhabited by only a few hardy animals. It is frequently referred to as the splash zone. Zone two extends from a height of +5.0 feet down to +2.5 feet, the level of mean higher low water, while zone three lies between the latter figure and the 0.0 tide line. Zone four is that area extending from 0.0 level downwards to the line of lowest low tide, about -1.5 feet. This area is uncovered for only a few hours during the month and is populated by animals and plants which are very sensitive to heating and desiccation.

Method of Examination

Of the three commonly used methods for determining the reproductive seasons of marine animals, two had to be discarded as being nonfeasible for this study. These two were the plankton analysis method for Acmaea larvae and the determination of spawning time by watching for settling larvae. The plankton analysis method, while of great applicability when masses of sessile, sub-littoral forms such as the oyster are being studied, is of little use here because it is impossible to distinguish between larvae of different species. This fact also militates against searching for newly settled and metamorphosed larvae, which method would be, in addition, very time consuming.

It thus became necessary to adopt a third procedure, that of periodic examination of samples of adult limpets collected from specific areas of the intertidal zone. The practice of collecting samples of the limpets at intervals of two weeks was initiated at the outset of the study. The dates of collection were chosen for the most favorable tidal conditions insofar as the daylight hours permitted. The coincidence of low tides with early morning hours in summer and with late afternoon hours in the winter months often made collections of very low intertidal forms difficult or impossible. In addition, the series of poor collecting tides during the spring and fall solstices often prevented these low intertidal collections. Occasionally a heavy surf would also intervene, and collecting would have to be restricted to the high and middle intertidal forms. Thus some gaps appear in the data which are the results of factors beyond our control. It must be admitted that while this method was quite adequate for establishing the yearly cycles of the several species, it is not adequate for determining the exact time of spawning by any one of the populations under study because of the fourteen-day interval between collections. In the cases of those species which live in the lower zones, i.e., three and four, collections could be made only on the lowest tides of the month. Here the method of study is limited by the natural tidal fluctuations which follow a fourteen-day cycle. However, those animals living at levels of +2.5 feet or higher could be collected almost daily, conditions of wind and surf permitting. Several factors made this procedure impractical. The nearest collecting areas are about thirty miles distant from the University and the time and expense involved in making the trip daily became enormous. The problem of storage in the laboratory had to be considered as well as the time available for examination of the specimens. For these reasons, then, the system of collecting as described above was decided upon as being the most suitable compromise.

The limpets were removed from their positions in the rocky intertidal, placed in pint collecting jars with sea water and returned to the laboratory. No effort was made to refrigerate the samples during the collecting trips. In the laboratory, the animals in their jars were placed in a refrigerator which was maintained at 11°C. and which was equipped with air outlets which permitted the jars of water to be aerated constantly. The jars were removed at intervals of two days, the water poured off, and the animals allowed temporarily to dry off and to reach room temperature for one to two hours. Fresh filtered sea water at room temperature was then added to the jars and the limpets returned to the refrigerator. The procedure was continued until time was available to examine the animals and to determine their reproductive condition.

The procedure for examination was as follows. The animal was removed from its shell by cutting the horseshoe-shaped shell muscle at its attachment to the shell. The entire visceral mass of the limpet is thus exposed, with the digestive gland uppermost. In Acmaea the single gonad lies between the dorsal surface of the foot and ventral surface of the digestive gland. It extends from the extreme posterior end of the animal anteriorly to the radula sac and sends dorsally a branch from the left side towards the right where it opens into the mantle cavity via the right kidney pore just to the right of the anus. When the animal is in a ripe condition, the gonad can be seen lying between the foot and the digestive gland. The animal is held in such a way that a dissecting needle can be inserted at the junction of the mantle with the posterior dorsal margin of the foot. The needle is then worked to the right and left around the foot, freeing the foot from the visceral mass while leaving the foot attached to the body anteriorly. The foot is flexed so that the ventral surface of the gonad can be examined under a dissecting microscope.

Since the individual animals varied considerably in size, the size of the gonad varied accordingly. Thus, absolute size of the gonad could not be taken as a criterion of reproductive condition when dealing with a sample of a species which showed great size variation. Acmaea asmi is an exception to this, since its size is not subject to much variation because of limitations placed upon this by its habitat upon Tegula funebralis. For this reason, the measurement of gonad thickness was used as a guide to the reproductive condition of the animals of this species. This measurement was made from the lateral view and included the distance from the ventral surface of the gonad to the loop of the intestine which marks the ventral extent of the digestive gland and the dorsal margin of the gonad. A small hand-scale graduated in millimeters was used for this purpose.

In the absence of any good quantitative measurements, a series of rather subjective terms was used to classify the various gonad conditions. The three conditions which are most readily determined are those classified as "ripe", "spawned", and "indeterminate". These terms are explained below.

In all ripe animals, both male and female, the most characteristic condition is that of extreme turgor of the gonad. When the mantle membranes are ruptured in such an animal, the gonad in its membrane bulges forth through the opening and, indeed, often ruptures so that the genital products themselves are exposed. This extreme turgor is readily recognized and serves as the best single characteristic of a ripe animal. In addition, the eggs of the female are seen to be in very tight clusters, so tight that the individual eggs are deformed from their normal spherical shape. The follicles of the male testis are completely filled with the white spermatic fluid and are uniformly opaque.

The spawned gonad of both sexes is characterized by being completely flaccid, the membrane of the emptied gonad having not recovered from the extreme distension of the ripe condition. In the female, a few residual eggs remain scattered loosely throughout the nearly empty sac and, in addition, a moderate quantity of granular material, probably young eggs, is present. The testis typically has on its surface a broad dark brown net which previously delimited the individual follicles. These may be almost empty and contain only small white or gray traces of spermatic fluid.

Animals in the indeterminate condition cannot be identified sexually. No traces of eggs or spermatic fluid are present and only microscopic examination of sections of the gonad would permit one to classify the animal as to its sex. This condition often follows the spawned condition in those limpets which spawn only once a year.

The two following conditions of the gonad are even more subjective in interpretation than the three mentioned above and are accordingly of less value.

An animal which is considered to be "partially ripe" is one whose gonad is being redeveloped from a previously spawned condition based on observations of the population under study. Typically, this term is applied as soon as the flaccid condition of the gonad disappears and it begins to fill with new gametes. It is used until the gonad assumes the extreme turgor of ripeness. In the female, the eggs are initially small and in loose clusters, gradually taking on the appearance described for the ripe ovary. The follicles of the testis slowly accumulate streaks of spermatic fluid in their centers and the follicles remain rather translucent until the testis is almost ripe.

In those populations where complete evacuation of the gonad contents does not occur at spawning, the gonad passes into a condition here referred to as "partially spawned". Obviously this state can be detected only when the previous sample was known to be ripe or almost so. The conversion from a ripe condition to one of partially spawned involves primarily a loss of the extreme turgor in both sexes. The eggs are large but in loose clusters or without arrangement. The follicles of the testis are clearly only partially filled with fluid.

Environmental Factors Analysed and Sources

Ocean water temperatures are those recorded by the U. S. Coast and Geodetic Survey at Fort Point, Presidio of San Francisco. These observations are made daily between 0700 and 0900 at the surface of the water.

Tidal heights are those of the Golden Gate, San Francisco, recorded by the U. S. Coast and Geodetic Survey. Although a correction factor of -0.2 feet should be applied to the open coast area of Princeton, Half Moon Bay, such corrections have not been made in this study.

Lunar cycles have been taken from the World Almanac.

The general yearly trends of these and other environmental factors may be outlined as follows.

Air temperature. The lowest mean monthly temperature is that of January from which level it rises steadily until June in monthly increments of two to three degrees Fahrenheit. From June until the maximum is reached in September there is a decline in the rate of temperature increase, the total rise during this period being only about two degrees. Beginning with October, there follows a rapid decrease in mean monthly temperature of about five degrees per month until the minimum of January is again reached. January and October are thus months characterized by reversals in the direction of temperature change.

Surface water temperatures. The mean surface water temperatures parallel closely those of the mean air temperatures. During the period of May through September, the air temperature means exceed that of the water, while the reverse is true during the other seven months.

Table 1

	Mean Monthly Air Temperature and Precipitation at San Francisco (source: World Almanac)		Mean Monthly Surface Water Temperature at Fort Point: 1922 - 1947 *)
	Temp. Degrees F	Precipit. (inches)	Temp. Degrees F
January	48	3.5	51
February	50	3.4	52
March	53	2.9	53.6
April	54	1.2	54.7
May	57	0.4	55.9
June	60	0.1	57.6
July	61	0.0	59.1
August	61	0.0	59.8
September	62	0 1	60.4
October	59	0 7	59.3
November	54	1.4	55.9
December	49	3.7	52.8

*) source: U. S. Coast and Geodetic Survey

January and October again are critical months.

Precipitation. The rainy periods are restricted, in the main, to the first five and the last three months of the year. The period of June through September has very little or no rain. These data are summarized in Table 1.

Spawning Patterns

Subgenus Acmaea

Acmaea mitra Eschscholtz, 1833

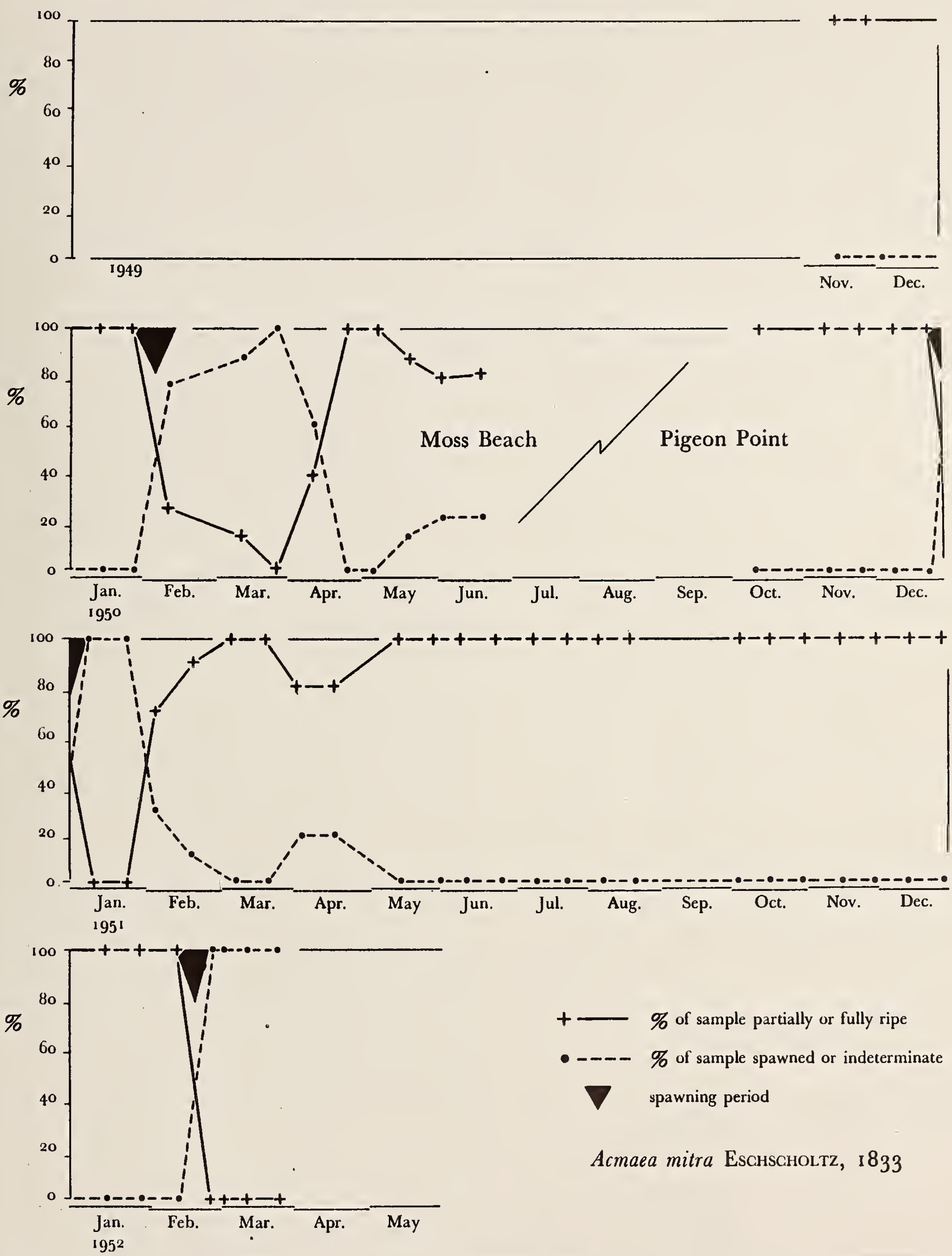
The ecology of this stenotopic species is interesting and quite distinctive. It is found exclusively on rocky substrata which are covered with the encrusting coralline algae Lithothamnion and Lithophyllum. These algae are common to lower zone three and zone four and are restricted not so much by their temperature requirements as by their need for almost continuous submergence judging from the fact that they are frequently found in permanent tide pools in zones two and three. Acmaea mitra is less tolerant of warm water and is found in greatest abundance at and below the zero tide level. The restriction of A. mitra to this type of substratum is based on the animals' utilization of coralline algae for food. The fecal pellets of the limpet are perfectly white calcareous strings composed of particles rasped from the plant. Occasionally a limpet of this species will be found on a rock in zone three which is not coralline encrusted. Such an abnormal position may be explained by the animal's having been torn loose from its usual position and cast upwards by the sea. Since such an animal does not void any fecal pellets, it is assumed that it is not feeding and will eventually starve to death.

The majority of the living material studied was taken from two areas: Moss Beach and Pigeon Point. Since this species has a very simple and clearcut reproductive cycle, it was possible to use only a few animals per collection and still obtain an accurate representation of the entire yearly cycle. This was fortunate, since at neither of the collecting sites are the animals abundant and heavy collections would soon completely destroy the populations. Typically, then, five animals were taken for examination at two-week intervals, the period of study extending from November, 1949, to the last of March, 1952. A total of 78 animals was taken from Moss Beach between November 19, 1949, and June 18, 1950. This total included 34 males, 28 females, and 16 indeterminate. Between October 14, 1950, and March 31, 1952, 221 animals were taken from Pigeon Point; 117 males, 100 females, and 4 indeterminate. Thus, in all, 299 limpets of this species were examined.

The annual nature of the reproductive cycle of this species at this latitude has been clearly established (Plate 10). It spawns in the localities under study once a year in the winter months. The approximate time of spawning is readily determined since the gonads of the animals have been in a state of extreme turgor for several months prior to the event, and the sudden appearance of spawned gonads is a striking change admitting of no misinterpretation. Another characteristic is a long and gradual buildup of the gonad which begins very soon after spawning has occurred. This redevelopment may begin either from an indeterminate or from a spawned gonad.

The spawning of the Moss Beach population was recorded in February and March of 1950. Although the major spawning took place between January 29 and February 12, it was not until March 28 that no unspawned animals were found, the total time elapsing between the onset and the completion of spawning being two months. By April 23 all of the animals in the samples were redeveloping the gonads, the small eggs being clearly visible in the ovary arranged in long sinuous rows. The decline of the percentage of ripe or partially ripe animals in May and June, 1950, does not represent a second spawning but rather the collection of several individuals which had either spawned later than the bulk of the population or else were retarded in the redevelopment of the gonad.

Two spawning periods were recorded from the Pigeon Point population. The first of these took place between December 22, 1950, and January 6, 1951. On the latter date the sample consisted entirely of partially spawned animals and it was not until the following collection on January 21 that completely spawned specimens were found. This spawning was thus completed in about a month and by March 4 the animals had begun gonad redevelopment. The decline in the number of partially ripe animals which is again seen during late March and April is caused by the collection of two animals which were indeterminate. By May 13 a period of redevelopment had begun which continued until the spawning of the following year.



Acmaea mitra ESCHSCHOLTZ, 1833